At page 4, line 32, after "ACLP", insert --(SEQ ID NO: 3 and SEQ ID NO: 4, respectively)--.

In the claims:

Please amend claim 7 as follows:

7. **(Twice Amended)** A method for identifying a gene which is up- or down-regulated during the differentiation of neural crest cells into smooth muscle cells, comprising

(i) culturing immortalized neural crest cells under culture conditions [wherein SM22α gene expression is induced for a time period] sufficient for the neural crest cells to [begin differentiation] <u>differentiate uniformly</u> into smooth muscle cells;

(ii) identifying genes which are up- or down-regulated under the culture conditions.

Please add new claims 31-33:

--31. The method of claim 7, wherein the immortalized neural crest cells include an oncogene.

32. The method of claim 31, wherein the immortalized neural crest cells include c-myc.

33. The method of claim 32, wherein the immortalized neural crest cells are Monc-1 cells.--

In the Figures:

Please replace originally filed Figures 6 and 9A with the enclosed copies of Figures 6 and 9A indicating appropriate SEQ ID Nos.

Remarks

Claims 1-30 are pending and claims 7-9 are currently under examination. Claim 7 has been amended. New claims 31-33 have been added. Support for the claim amendments and new claims can be found throughout the specification. For example, support for "uniformly" can be found at page 11, lines 25-28. Support for new claims can be found, e.g., at page 11, lines 31-32 and at page 12, line 4. No new matter has been added.

Amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite

prosecution of the present application. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Sequence Requirements

Applicants submit that a Sequence Listing and Diskette have been submitted on January 24, 2000. The specification has been amended to indicate the SEQ ID Nos in the Brief Description of figures 6 and 9. Also filed herewith are copies of Figures 6 and 9A, on which the appropriate SEQ ID Nos are indicated in red (pursuant to 37C.F.R. 1.121(d)) for subsituting with Figures 6 and 9A as originally filed. Accordingly, the application is believed to be in accordance with 37 C.F.R. 1.821(a)(1) and (a)(2).

Priority

The specification has been amended to recite the claim of priority to the three provisional applications.

Election/Restriction

It is the Examiner's position that newly submitted claims 12-21 are directed to an invention that is independent or distinct from the invention originally claimed, because "the claims are no longer drawn to the same functional activity, previously regulating proliferation or migration, but are now drawn to genes which are up-regulated or down-regulated during differentiation which differs from the function of an agent which modulates this response." Applicants respectfully traverse this statement.

In response to the Restriction Requirement dated March 17, 2000, Applicants elected Group II, claims 7-9 and 12-21, which, as stated in the Restriction Requirement are "drawn to a method for identifying a gene." Thus, the Restriction Requirement was not based on functional activity of the genes identified. Accordingly, amending the claims to recite "modulation of differentiation of neural crest cells" instead of "modulation of proliferation and/or migration" should not bring claims 12-21 outside of Group II having claims drawn to a method for identifying a gene.

Applicants also note that, contrary to the Examiner's statement, claims 12-21 were not newly added. These claims were merely amended.

Thus, reconsideration of the withdrawal of claims 12-21 from consideration as being directed to a non-elected invention is respectfully requested.

Rejection of claims 7- under 35 U.S.C 101

Claims 7-9 were rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific or substantial, credible asserted utility or a well established utility. Applicants respectfully traverse this rejection.

> The Examiner states that,
> the methods and research steps merely constitute research and reagents utilized by the skilled artisan to perform further experimentation to discover a "real-world" use of the specified in vitro system. The recited uses also do not constitute a credible or well-established utility because the invention does not disclose specific and substantial uses for any particular identified sequence using the general experimental method. In addition, the specification does not teach any method for determining the use of the identified compounds which are modulated during neural crest cell smooth muscle differentiation.

Applicants respectfully traverse these statements.

Applicants disclose at least one utility that is specific, substantial and credible, which is also a well-established utility. The specification discloses, e.g., that genes identified as being up- or down-regulated during differentiation or migration of smooth muscle cells are useful as diagnostic markers (see, e.g., page 7, lines 10-13). As further described in the specification, and as was well known in the art at the time the application was filed, dedifferentiation of smooth muscle cells results in numerous diseases, e.g., occlusive arteriosclerotic diseases, atherosclerosis, heart attacks, strokes, restenosis, and hypertension (paragraph bridging pages 1 and 2; and page 45, lines 9-23). Accordingly, genes that are up- or down-regulated during differentiation of neural crest cells into smooth muscle cells can be used as markers of the stage of differentiation of these cells, and thus, as diagnostic markers, for predicting whether a subject is likely to develop, e.g., an occlusive arteriosclerotic disease. For example, the presence in vascular cells of a subject of mRNA or protein of a gene which is down-regulated during differentiation would indicate that a dedifferentiation of the smooth muscle cells is occurring and that, therefore, the subject may be at risk of developing a disease associated with dedifferentiation of smooth muscle cells.

Applicants note that the use of the genes identified by the claimed method as diagnostic markers constitute a specific activity, since these genes can be used as markers for specific diseases, not merely any disease. The claimed utility is also substantial, as it defines a "real world" use, which does not constitute carrying out further research to identify or reasonably confirm a "real world" context. The claimed utility would also have been credible to a person of skill in the art at the time the invention was made, based on the disclosure and the knowledge generally available to a person of skill in the art at the time the invention was made. Applicants

further submit that the claimed utility is also a well-established utility, since (1) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., identification of genes which are up- or down-regulated during differentiation of neural crest cells into smooth muscle cells); and (2) the utility is specific, substantial and credible.

Thus, reconsideration and withdrawal of the rejection of claims 7-9 under 35 U.S.C. 101 is respectfully requested.

Rejection of claims 7-9 under 35 U.S.C. 112, first paragraph

Claims 7-9 were rejected under 35 U.S.C. 112, first paragraph, allegedly because, "since the claimed invention is not supported by either a specific and substantial, asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention." Applicants respectfully traverse this rejection.

As set forth above, the specification discloses at least one credible assertion of specific and substantial utility for the claimed invention to satisfy the utility requirement. The specification discloses that genes which are up- or down-regulated during differentiation of neural crest cells into smooth muscle cells can be used as diagnostic markers. The specification further describes that gene expression, e.g., gene expression of diagnostic markers, can be measured by various techniques, e.g., Northern blots (see, e.g., page 56, lines 3-13), *in situ* hybridization (see, e.g., page 57, lines 28-34), and Western blots (e.g., page 56, lines 12-27). Thus, a person skilled in the art would know how to use the claimed invention.

The Examiner also states that "[t]he specification and claims do not teach SM22α, levels or time periods of induction such that the skilled artisan can determine if SM22α is induced for a time period sufficient for the neural crest cells to begin differentiation into smooth muscle cells and thus the skilled artisan can not readily make and use the culture system." The Examiner concludes that "[t]hus the skilled artisan is unable to determine without further direction those requirements necessary for SM22α induction and differentiation of neural crest cells to smooth muscle cells." Applicants respectfully traverse these statements.

The specification discloses that SM22α is a marker of smooth muscle cells, and that expression increased during differentiation of neural crest cells into smooth muscle cells. The specification teaches, e.g., at page 16, lines 14-18, "[a]nother well studied maker of smooth muscle cell is the SM22α gene, which is expressed exclusively in vascular and visceral smooth muscle cell in adult animals. (Kim, et al. (1997) Mol. Cell. Biol. 17, 2266-2278). As with the smooth muscle α-actin and calponin mRNAs, expression of the SM22α mRNA increased after incubation in SMDM as shown in Figure 3." Page 24, lines 15-17, states that "SM22α is active

only in differentiated rat aortic smooth muscle cells or Monc-1 cells, but is inactive in dedifferentiated rat aortic smooth muscle cells or undifferentiated Monc-1 cells." Page 49, lines 17-20, indicate that "[t]he activity of SM22 promoter was low in Monc-1 cells. Differentiation of these cells into SMC caused a 20-30 fold increase in the activity of the wild-type SM22 promoter."

The specification further discloses medium that can be used to differentiate neural crest cells, e.g., Monc-1 cells, into smooth muscle cells (see, e.g., at page 50, lines 16-20). The specification also indicates the time necessary for differentiation, e.g., 4 days (see, e.g., page 3, lines 21-22). Thus, the specification provides sufficient guidance for a person of skill in the art, at the time the application was filed, to differentiate neural crest cells, e.g., Monc-1 cells, into smooth muscle cells and thereby obtain an increase in SM22α gene expression, without undue experimentation.

Applicants note that claim 7 has been amended to delete reference to SM22α. The claim amendment is not to be interpreted as an acquiescence of any of the Examiner's rejection. This claim amendment was made to more specifically claim what the inventors regard as their invention. In fact, differentiation of neural crest cells into smooth muscle cells can be evidenced by monitoring a marker other than SM22α, as well as by monitoring the morphology of the cells (see, e.g., page 49, lines 14-17, and Fig. 1A (photo of undifferentiated Monc-1 cells) and Fig. 1B (photo of Monc-1 cells differentiated into smooth muscle cells).

Thus, reconsideration and withdrawal of the rejection of claims 7-9 under 35 U.S.C. 112, first paragraph, is respectfully requested.

Rejection of claims 7-9 under 35 U.S.C. 112, second paragraph

Claims 7-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiners states that "[t]he specification and claims do not teach the metes and bounds of SM22α, level or time period of induction such that the skilled artisan can determine if SM22α is induced in cells and such that SM22α is induced for a time period sufficient for the neural crest cells to begin differentiation into smooth muscle cells and thus the skilled artisan can not readily determine the metes and bounds of the claims." Applicants respectfully traverse this rejection.

As described above, the specification teaches conditions (including time and medium) for inducing differentiation of neural crest cells, e.g., Monc-1 cells, into smooth muscle cells. In addition, claim 7 has been amended by deleting reference to SM22α.

Accordingly, reconsideration and withdrawal of the rejection of claims 7-9 under 35 U.S.C. 112, second paragraph, is respectfully requested.

Rejection of claim 7 under 35 U.S.C. 102(b) as being anticipated by Shah et al.

Claim 7 was rejected under 35 U.S.C. 102(b) as being anticipated by Shah et al. (1996) *Cell* 85: 331. Applicants respectfully traverse this rejection.

Shah et al. is relied upon by the Examiner as teaching "mRNA analysis of neural crest cells which are immortalized to the extent that they are capable of multipotent proliferation, and are differentiated to smooth muscle cells."

A claim is anticipated by a reference if the references teaches each and every element of the claims. Applicants respectfully submit that Shah et al. fail to disclose immortalized neural crest cells. Shah et al. disclose that the rat neural crest cells used in the experiments described in the reference, were isolated and cultured as described (Stemple and Anderson (1992) *Cell* 71:973), with minor modifications (bottom left column of page 341 of Shah et al.). Shah et al. state "the present study represents the first case in which de novo differentiation of these cells from a naturally occurring precursor has been elicited in vitro" (top of the left column at page 341; emphasis added). Thus, the cells described in Shah et al. are not immortalized cells.

Thus, reconsideration and withdrawal of the rejection of claim 7 under 35 U.S.C. 102(b) as being anticipate by Shah et al. is respectfully requested.

Rejection of claim 7 under 35 U.S.C. 102(e) as being anticipated by Anderson et al., U.S. Patents 5,672,499 and 6,001,654

Claim 7 is rejected under 35 U.S.C. 102(e) as being anticipated by Anderson et al. U.S. Patents 5,672,499 and 6,001,654. Applicants respectfully traverse this rejection.

Anderson et al. are relied upon by the Examiner as teaching "O cells which are smooth muscle cells derived from immortalized neural crest cells and which are recognized to differentially express smooth muscle actin, desmin and calponin in comparison to precursors or neural stem cells differentiated to neurons or glia, as evidenced by antigen expression, see in particular abstract and Example 10."

A claim is anticipated by a reference if the references teaches each and every element of the claims, and the reference is enabling. Applicants respectfully submit that neither of the Anderson et al. references discloses differentiation of immortalized neural crest cells into smooth muscle cells. Although the references contain a prophetic example indicating how one could immortalize neural crest stem cells, the references fail to provide any evidence that such

procedures would result in immortalized neural crest cells, and the reference further fails to disclose conditions for differentiation of such immortalized neural crest cells into smooth muscle cells. In addition, the references fail to teach methods for uniformly differentiating neural crest cells into smooth muscle cells. See, e.g., Example 15 of the '654 patent, which describes the identity of the cells in the mixed cell populations obtained from differentiating neural crest stem cells. On the contrary, the methods disclosed by Applicants permit differentiation of neural crest cells into a population of cells in which almost 100% of the cells are smooth muscle cells (see, e.g., page 11, lines 18-28 of the specification).

Thus, reconsideration and withdrawal of the rejection of claim 7 under 35 U.S.C. 102(e) as being anticipate by Anderson et al. U.S. Patents 5,672,499 and 6,001,654 is respectfully requested.

Rejection of claims 7-9 under 35 U.S.C. 103(a)

Claims 7-9 were rejected under 35 U.S.C. 103(a) as being unpatentable over Shah et al. (*Cell* 85:331 (1996); Anderson et al. U.S. Patent 5,672,499; Anderson et al. U.S. Patent 6,001,654, Baetscher et al. U.S. Patent 5,922,601 and Liang et al. U.S. Patent 5,599,672. Applicants respectfully traverse this rejection.

It is the Examiner's position that "[i]t would have been *primae facie* obvious to the skilled artisan as motivated by Baetscher and Liang to study the up and down-regulation of genes in *in vitro* culture systems which have been shown to possess genetic loci which are differentially expressed in the differentiated muscle cells of Shah or Anderson et al. as set forth above utilizing the modified technology of either Baetscher et al. or Liang et al."

As set forth above, Applicants respectfully submit that neither Shah et al., nor either of the Anderson et al. references teach or suggest immortalized neural crest cells which differentiate almost uniformly into smooth muscle cells. Since neither Baetscher nor Liang cure this defect, the cited references fail to teach or suggest all claim limitations.

Thus, reconsideration and withdrawal of the rejection of claims 7-9 under 103(a) is respectfully requested.

Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,

By: _____

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